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TECHNICAL MANUSCRIPT 495

SURVIVAL OF MICROORGANISMS ON COVERED CONTAMINATED STAINLESS STEEL AFTER 2½ YEARS

Dorothy M. Portner
Robert K. Hoffman
Charles R. Phillips

DECEMBER 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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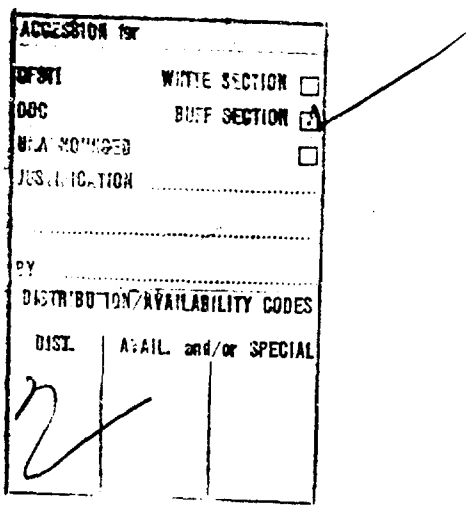

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Physical Defense Division
COMMODITY DEVELOPMENT & ENGINEERING LABORATORIES

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ABSTRACT

Death rates for a 2½-year period were determined for microorganisms on stainless steel strips that were initially contaminated by handling or by aerial fallout. After 2 weeks' storage, the level of contamination from handling had decreased 94%, and after 2½ years most but not all of the organisms were dead. Organisms accumulated on stainless steel from aerial fallout survived longer; 40% died in 2 weeks and 93% in 2½ years. The results are also discussed in relation to the "plateau" phenomenon.

I. INTRODUCTION

As part of the overall investigation of spacecraft sterilization, this study was undertaken to determine the death rate of naturally accumulated microorganisms on stainless steel strips. The probability of sterilizing a spacecraft by any method is enhanced when the initial microbial contamination is minimal. We observed that the number of microorganisms accumulated on stainless steel surfaces from aerial fallout rapidly reached a level that did not appear to increase significantly thereafter, even during a long exposure period. This "plateau" phenomenon was then further studied and confirmed in a year-long study[1] conducted in clean rooms, but the number of organisms or level of the plateau was lower than that observed in adjacent areas. The plateau phenomenon was also observed in subsequent clean room studies.[2,3] The plateau level is a function of the amount of airborne contamination in the area. Thus, a spacecraft constructed and assembled in a clean room would have a lower level of contamination than a craft assembled in a factory area.

Since there is a continual accumulation of microorganisms on a surface from aerial fallout, there must be an equivalent death rate for the plateau phenomenon to exist. If, then, a contaminated surface is covered to prevent additional microbial accumulation, sterility should result in time. Others have studied[3] the death rate over a 3-week period for microorganisms originally accumulated on stainless steel from natural fallout and from handling. In the study cited, 50% of the fallout organisms and 90% of the organisms from handling died during the 3 weeks' storage. The extensive study reported here shows the rate of decrease of viable microorganisms on stainless steel strips initially contamined by handling or by aerial fallout and then covered and assayed after storage periods of 2 weeks to 2½ years.

II. MATERIALS AND METHODS

For each test, 80 sterile stainless steel strips (1 by 2 inches) were contaminated. First, a tray of 40 strips, placed on a shelf in the laboratory, were exposed to aerial fallout for 22 days. Then the other 40 sterile strips were well-fingered for a few seconds by five persons. Five strips contaminated by aerial fallout and five by handling were assayed to obtain the initial contamination level. The 70 remaining strips were placed in sterile petri dishes and stored in a laboratory cabinet from 2 weeks to about 2½ years before being assayed. Six replicate tests involving a total of 480 stainless steel strips were conducted at intervals

over a 4-month period. By staggering the intervals for assaying the strips, the storage periods of 2, 8, 16, 24, 32, and 52 weeks were the same for all tests. The final assay, however, was not similarly staggered because after such an extended storage period the differences in the storage time among the tests were assumed to be negligible. The final storage period varied from 129 to 143 weeks.

The remaining viable microbial population on the steel after each storage period was determined for five strips contaminated by aerial fallout and for five contaminated by handling. A steel strip was placed in a bottle containing 50 ml of 0.05% Tween-20 solution and shaken mechanically for 5 minutes. Then 25 ml (half of the sample) were placed in petri dishes and pour plates prepared with tryptose agar. Plate counts were made after 72 hours' incubation at 98 F. For the final storage period (129 to 143 weeks), the entire 50-ml sample and the strip were placed in petri dishes and then pour plates were prepared to establish the degree of contamination.

III. RESULTS AND DISCUSSION

The number of microorganisms recovered from 30 contaminated stainless steel strips is shown for each storage period in Table 1. The 30 steel strips represent the total number of strips used for the six replicate tests for one storage period. The total rather than the average recovery of six tests per storage period is given because it seemed more meaningful to express the data in whole numbers rather than in fractions. The strips were not uniformly contaminated initially by either method. With the maximum recoveries of only a few hundred microorganisms per strip, the variation among the strips contaminated by aerial fallout was tenfold; a 100-fold variation was observed among the strips that were handled. No microorganisms were recovered from two of the strips that were assayed within a short period after handling. After storage, no microbial recovery was obtained for many of the strips assayed. The frequency of this occurrence is also shown in Table 1.

Approximately the same initial level of contamination was obtained by both handling and aerial fallout, but the survival after storage was markedly different. After 2 weeks' storage, the contamination level from handling was reduced 94%, but the reduction in the contamination level from aerial fallout was only about 40%, and 7% of the viable organisms initially accumulated from aerial fallout survived 2½ years of storage. Twenty-six of the 30 strips initially contaminated by handling were sterile after 2½ years, but the fact that four strips were not sterile indicates that a surface covered after handling would not necessarily be sterile after a reasonable storage time.

TABLE 1. MICROBIAL RECOVERY FROM STAINLESS STEEL INITIALLY CONTAMINATED BY HANDLING OR FALLOUT AND THEN COVERED AND STORED FOR VARIOUS PERIODS

Storage, weeks	Handling[a]		Fallout[b]	
	Total Number Organisms Recovered[c]	No. Samples Plated Showing No Organisms[c]	Total Number Organisms Recovered[c]	No. Samples Plated Showing No Organisms[c]
0	1,640	2	1,946	0
2	92	16	1,162	0
8	46	20	656	0
16	30	19	526	0
24	24	21	522	1
32	18	24	426	0
52	26	22	298	1
129-143	6	26	132	3

a. All samples handled by five persons.
b. Samples exposed to aerial fallout for 22 days.
c. Based on 30 determinations.

No attempt was made to identify any of the microorganisms recovered. However, the rapid decrease in the level of contamination on steel that was handled suggested a population composed mainly of non-sporeforming bacteria. Studies in other laboratories[3] identified most of the microorganisms deposited on steel strips by handling as non-sporeformers. They also reported that 80% of the organisms died during a 2-week period at constant temperatures and relative humidity (RH). The results given here show the same magnitude of reduction after 2 weeks' storage in an uncontrolled ambient environment. During contamination and subsequent storage, the steel strips were subjected to seasonal environmental changes in temperature (60 to 90 F) and RH (20 to 80%). A study of the effect of ambient temperature and RH upon the survival of a heterogeneous population on a surface contaminated by handling or aerial fallout would not necessarily be meaningful because the type and the number of microorganisms composing the heterogeneous population would differ greatly from one location to another and from one time to the next. Furthermore, the protection afforded to microorganisms by oils or other materials from the hands, dust, etc., varies greatly.

The results suggest that the apparent plateau is probably not a true plateau of numbers of viable organisms accumulated on surfaces as it appeared to be in the samples exposed to aerial fallout for several weeks and as long as a year as previously reported.[1] There must be a gradual increase in viable count over a much longer time because of the accumulation of hardy sporeformers. Even these have a death rate, however, so eventually a true plateau must be reached. The reason for an apparent plateau occurring within a few weeks is that microorganisms accumulate quite readily on exposed surfaces but a great percentage of these are cells that die rapidly. Within a short time the death rate approaches the collection rate, but even so a gradual upward trend will continue because of the accumulation of sporeformers that die slowly. This is a gradual trend that is hidden by the great variation in numbers of organisms that accumulate concurrently on surfaces, even next to one another. Previous studies in this laboratory showed this variation to be almost 100-fold.

In this study, after 2½ years' storage a 15-fold decrease in population occurred on steel initially contaminated by aerial fallout. Most, but not all, of the population died on steel contaminated by handling. The "die-off" of microorganisms on surfaces contaminated by aerial fallout or handling was characterized by an initial rapid death rate which was followed by a very slow death rate of the remaining few. The results indicate that the death of naturally accumulated microorganisms on surfaces is insufficient to produce a sterile spacecraft in a reasonable time when the constructed craft has merely been covered with a shroud and allowed to stand.

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13. ABSTRACT

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14. Key Words

Microbial contamination
Death rate
Storage
Stainless steel surface
Handling
Aerial fallout

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